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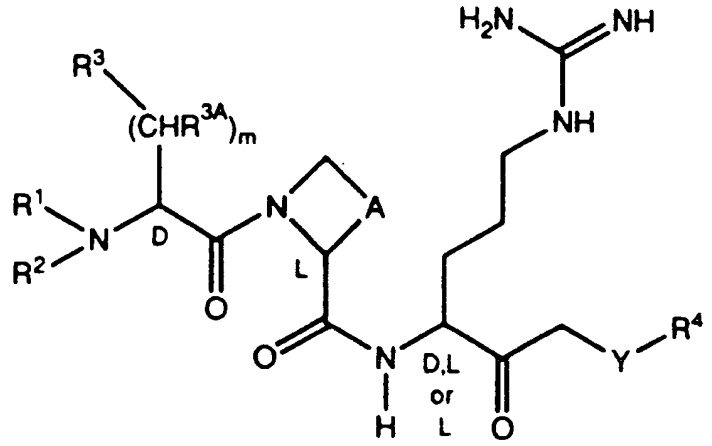
(54) New isosteric peptides.

(57) The invention relates to new competitive inhibitors of thrombin, their synthesis, pharmaceutical compositions containing the compounds as active ingredients, and the use of the compounds as anticoagulants for prophylaxis and treatment of thromboembolic diseases, according to the formula



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wherein :

A represents $-CH_2-$, $-CH=CH-$, $-CH_2-CH_2-$ or $-CH_2-CH_2-CH_2-$;

R^1 and R^2 are the same or different and each represents H or X-B-, where B is a straight or branched alkylene group having 1-3 carbon atoms and X is H, methyl, ethyl, a cycloalkyl group having 3-6 carbon atoms or R'CO-, where R' is OH, a straight or branched alkoxy group having 1-4 carbon atoms, NH_2 or NHR'', where R'' is a straight or branched alkyl group having 1-4 carbon atoms, or X is a carboxylic acid mimic, known per se, selected from $-PO(OR''')_2$, $-SO_3H$ and 5-(1H)-tetrazolyl, and R''' is H, methyl or ethyl, or B is $-SO_2-$ and X is methyl or ethyl ;

m is 0, 1 or 2, R^3 represents a cyclohexyl group and R^{3A} represents H : or

m is 1 and R^3 represents a cyclohexyl or phenyl group and R^{3A} forms an ethylene bridge together with R^1 ;

Y represents O or $S(O)_p$, where p is 0, 1 or 2 ;

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R^4 represents H ; a straight or branched alkyl or a cycloalkyl having 1 to 6 carbon atoms unsubstituted or substituted with one or more fluoro atoms and/or substituted with a phenyl group : a substituted or unsubstituted aromatic ring selected from phenyl, 4-methoxy-phenyl, 4-tertiary-butyl-phenyl, 4-methyl-phenyl, 2-, 3- or 4-trifluoro-methyl-phenyl, phenyl substituted with 1-5 fluoro atoms : or $-CH(CF_3)$-phenyl, either as such or in the form of a physiologically acceptable salt and including stereoisomers.

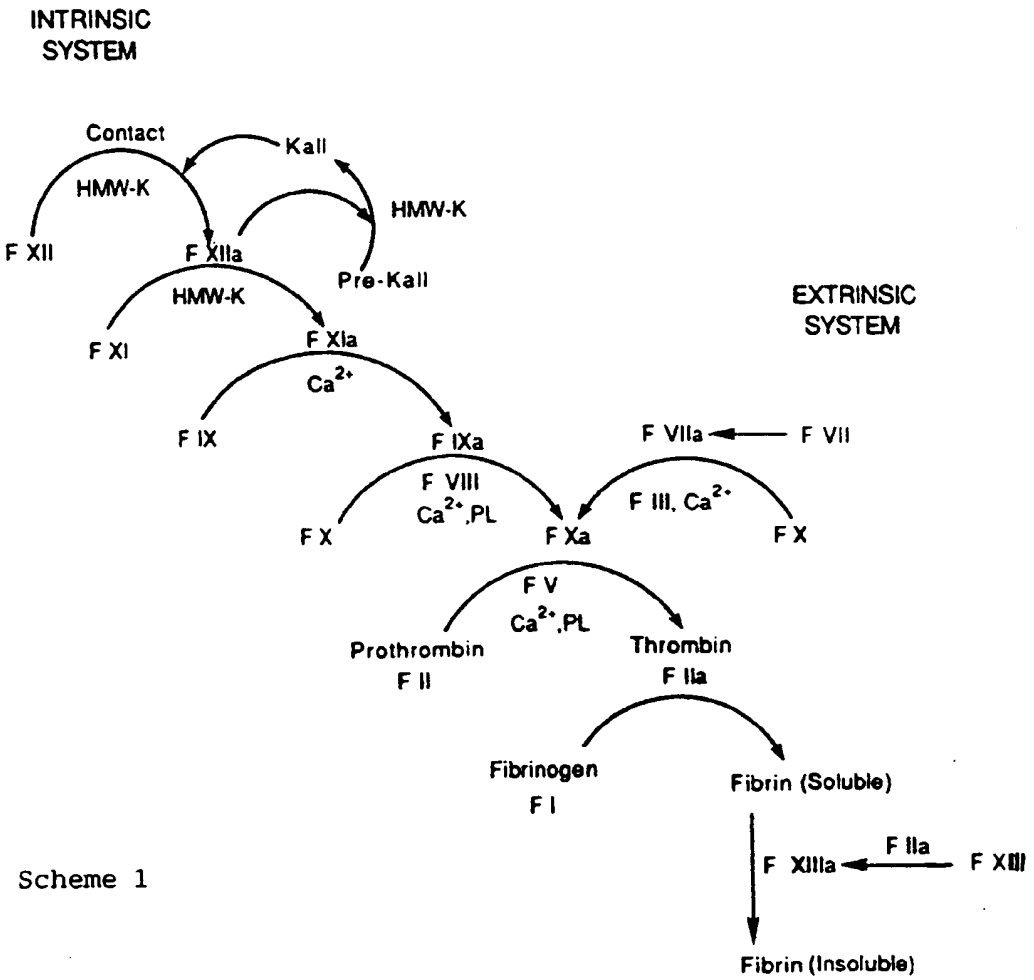
This invention relates to new competitive inhibitors of thrombin, their synthesis, pharmaceutical compositions containing the compounds as active ingredients, and the use of the compounds as anticoagulants for prophylaxis and treatment of thromboembolic diseases such as venous thrombosis, pulmonary embolism, arterial thrombosis, in particular myocardial infarction and cerebral thrombosis, general hypercoagulable states and local hypercoagulable states, e.g. following angioplasty and coronary bypass operations.

BACKGROUND

Blood coagulation is the key process involved in both haemostasis (i.e. prevention of blood loss from a damaged vessel) and thrombosis (i.e. the pathological occlusion of a blood vessel by a blood clot). Coagulation is the result of a complex series of enzymatic reactions outlined in the Scheme 1 below where the various clotting factors are designated by Roman numerals.

Thrombin plays a central role in coagulation, whether the process is initiated by the intrinsic or extrinsic pathways: it activates platelets, it converts fibrinogen into fibrin monomers, which polymerise spontaneously into filaments, and it activates FXIII, which in turn crosslinks the polymer to insoluble fibrin. Thrombin further activates FV and FVIII in a positive feedback reaction.

Inhibitors of thrombin are therefore expected to be effective anticoagulants.



Scheme 1

PRIOR ART

The first inhibitors of thrombin based on electrophilic ketones were developed as described by M. Szelke and D.M. Jones in EP-A1-0,118,280, GB priority date 4th March 1983. These earlier compounds were derived

from the P3 - P2' pentapeptide sequence of the fibrinogen Aα chain in which the scissile P_1 - P_1' peptide bond was replaced with the -CO-CH_2-moiety, forming a keto isostere to the corresponding peptides.

Other known examples of serine proteinase inhibitors based on electrophilic ketones include the following:

(a) M. Kolb et al. (Merrel-Dow) EP-A2-0,195,212 (Priority date 4.2.86) describing peptidic α-keto esters and amides,

(b) B. Imperiali and R.H. Abeles, Biochemistry 1986. 25. 3760 (peptidyl fluoroalkyl ketones),

(c) Ueda et al., Biochem. J. 1990. 265. 539 (peptidyl fluoroalkyl ketones).

(d) D. Schirlin et al. (Merrel-Dow) EP-A1-0,362,002 (priority date 1.9.88) describing fluoroalkylamide ketones.

(e) P. Bey et al. (Merrel-Dow) EP-A2-0,364,344 (priority date 7.10.88) describing α,β,δ-triketo compounds.

(f) E.N. Shaw et al. (Research Corporation) US-4,318,904 (priority date 25.04.80) describing peptide chloro-methyl ketones e.g. H-DPhe-Pro-Arg-CH_2Cl

Inhibitors of thrombin based on peptide aldehydes have been reported by S. Bajusz et al. in J. Med. Chem. 1990. 33. 1729, and in (Richter Gedeon Vegyeszeti Gyar R T) EP-A2-0,185,390 (priority date 21.12.84).Thrombin inhibitors as peptides comprising C-terminal boronic acid derivatives of arginine and isothiouronium analogues thereof have been reported by A.D. Kettner et al. (Du Pont) EP-A2-0,293,881 (priority dates 5.6.87 and 6.4.88).

There are examples of thrombin inhibitory arginine derivatives or analogues not containing an electrophilic ketone, e.g.:

(a) S. Okamoto et al. (Mitsubishi Chemical Industries Ltd.) EP-A1-0,008,746 (priority date 31.08.78) describing arylsulphonyl arginine amides e.g. argatroban.

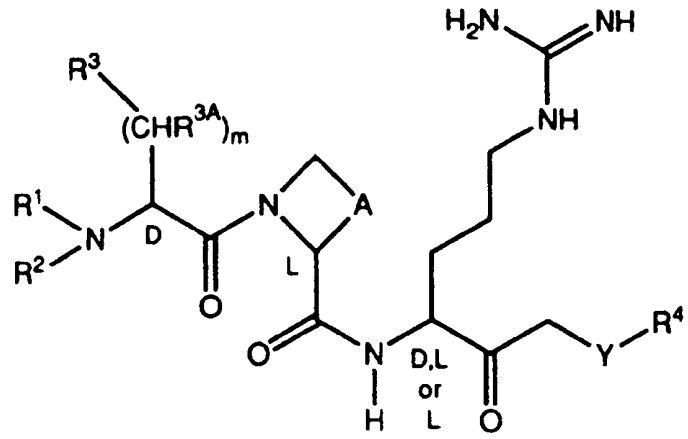
(b) J. Stürzebecher et al., Pharmazie 1981. 36. 639 (arylsulphonyl p-amidinophenylalanine amides).

An object of the present invention is to provide novel and potent thrombin inhibitors with competitive inhibitory activity towards their enzyme i.e. causing reversible inhibition. A further object is to obtain inhibitors which are orally bioavailable and selective in inhibiting thrombin over other serine proteases. Stability, duration of action, and low toxicity at therapeutic dosages are still further objects of the invention.

DISCLOSURE OF THE INVENTION

Compounds

It has been found that compounds of the general formula 1, either as such or in the form of physiologically acceptable salts and including stereoisomers, are potent inhibitors of thrombin:



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In formula 1, and when occurring below unless specified otherwise, the following applies:

A represents -CH_2-, -CH=CH-, -CH_2-CH_2- or -CH_2-CH_2-CH_2- ;

R^1 and R^2 are the same or different and each represents H or X-B-, where B is a straight or branched

alkylene group having 1-3 carbon atoms and X is H, methyl, ethyl, a cycloalkyl group having 3-6 carbon atoms or R'CO-, where R' is OH, a straight or branched alkoxy group having 1-4 carbon atoms, NH_2 or NHR", where R" is a straight or branched alkyl group having 1-4 carbon atoms, or X is a carboxylic acid mimic, known per se, selected from $-PO(OR"')_2$, $-SO_3H$ and 5-(1H)-tetrazolyl, and R"' is H, methyl or ethyl, or B is $-SO_2-$ and X is methyl or ethyl;

m is 0, 1 or 2, R^3 represents a cyclohexyl group and R^{3A} represents H; or

m is 1 and R^3 represents a cyclohexyl or phenyl group and R^{3A} forms an ethylene bridge together with R^1;

Y represents O or $S(O)_p$, where p is 0, 1 or 2;

R^4 represents H; a straight or branched alkyl or a cycloalkyl having 1 to 6 carbon atoms unsubstituted or substituted with one or more fluoro atoms and/or substituted with a phenyl group; a substituted or unsubstituted aromatic ring selected from phenyl, 4-methoxy-phenyl, 4-tertiary-butyl-phenyl, 4-methyl-phenyl, 2-, 3- or 4-trifluoro-methyl-phenyl, phenyl substituted with 1-5 fluoro atoms; or $-CH(CF_3)$-phenyl.

Compounds of formula 1 relate to the peptide sequence of human fibrinogen Aα chain representing modified subsites P_3 - P_1':

```
H-Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-
  1                  5                   10

                  P3  P2  P1  ! P1' P2' P3'
-Glu-Gly-Gly-Gly-Val-Arg----Gly-Pro-Arg-Val-
                  14  15  16    17        20
```

This sequence is identified as SEQ ID NO: 1 in the Sequence Listing.

According to a preferred embodiment the invention relates to compounds of Formula 1, wherein

A represents $-CH_2-CH_2-$ or $-CH_2-CH_2-CH_2-$;

R^1 is H and R^2 represents $HOCO(CH_2)_n-$ or $CH_3CH_2OCO(CH_2)_n-$, and n is 1 or 2;

Y is O

m is 1, R^3 represents a cyclohexyl group and R^{3A} represents H;

Particularly advantageous embodiments of the invention are represented by the compounds:

$HOOC-CH_2$-DCha-Pro-Arg-CH_2-O-CH_2-CF_3

$HOOC-CH_2$-DCha-Pic-Arg-CH_2-O-CH_2-CF_3

$HOOC-CH_2$-DCha-Pic-Arg-CH_2-O-nBu

$HOOC-CH_2-CH_2$-DCha-Pro-Arg-CH_2-O-nBu

$HOOC-CH_2-CH_2$-DCha-Pro-Arg-CH_2-O-CH_2-CF_3

$HOOC-CH_2$-DCha-Pro-Arg-CH2-O-nBu

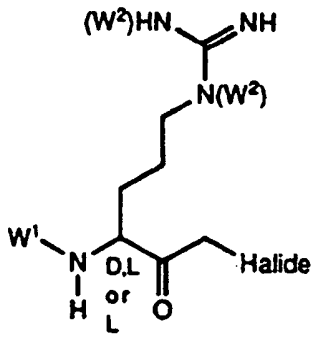
Medical and pharmaceutical use

In a further embodiment the invention relates to treatment, in a human or animal organism, of conditions where inhibition of thrombin is required. The compounds of the invention are expected to be useful in particular in animals including man in treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues. Disease states in which the compounds have a potential utility, in treatment and/or prophylaxis, include venous thrombosis and pulmonary embolism, arterial thrombosis, such as in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis. Further, the compounds have expected utility in prophylaxis of atherosclerotic diseases such as coronary arterial disease, cerebral arterial disease and peripheral arterial disease. Further, the compounds are expected to be useful together with thrombolytics in myocardial infarction. Further, the compounds have expected utility in prophylaxis for reocclusion after thrombolysis, percutaneous transluminal angioplasty (PTCA) and coronary bypass operations. Further, the compounds have expected utility in prevention of rethrombosis after microsurgery. Further, the compounds are expected to be useful in anticoagulant treatment in connection with artificial organs and cardiac valves. Further, the compounds have expected utility in anticoagulant treatment in haemodialysis and disseminated intravascular coagulation. The daily dosage will normally be within 0.1 mg to 10 g of active ingredient. Intravenous solutions preferably contain 0.1-100 mg/ml, while a dosage unit preferably contains 1-1000 mg and is preferably administered 1-4 times a day.

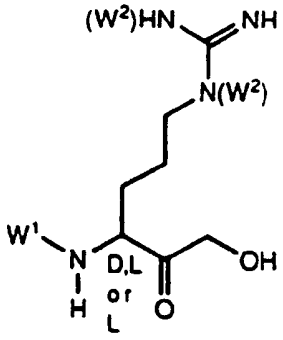
A further expected utility is in rinsing of catheters and mechanical devises used in patients in vivo, and as an anticoagulant for preservation of blood, plasma and other blood products in vitro.

Preparation

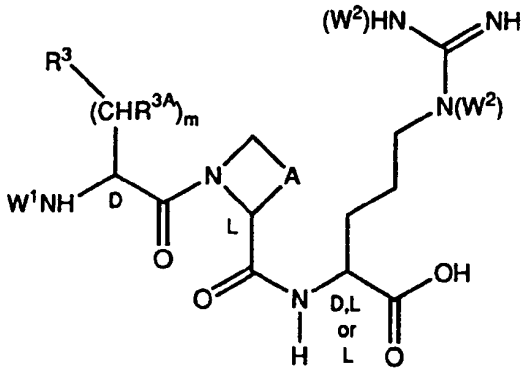
A further objective of the invention is the mode of preparation of the compounds of the invention. Thus the invention further relates to a process for preparation of compounds according to formula 1, which process comprises
(method I) displacement by R^4Y^- (Y=O,S) of the halogen of a halomethylketone of the formula



wherein W^1 is an amino terminal protecting group such as tertiary butoxycarbonyl and W^2 is a protecting group such as benzyloxy carbonyl, (as illustrated in Procedures (A), (D) and (F)), reduction of the ketone to alcohol, removal of the amino terminal protecting group, standard peptide coupling, followed by oxidation of the alcohol, giving the protected tripeptide ketone, removal of the amino terminal protecting group, followed by N-alkylation, (as illustrated in Procedures (A), (B), (G) and (H)), and deprotection, or replacing a protected dipeptide in the coupling reaction referred to above and illustrated (eg. Procedure (A)(iii)), with an amino-terminal-N-alkylated-N-trifluoroacyl-protected dipeptide (Procedure K), followed by oxidation and deprotection, or (method II) alkylation, with an R^4-halide, of an α-ketol of the formula



wherein W^1 and W^2 are as defined above, (as illustrated in Procedure (E)), and then further reacting as in method I, or (method III) by using the modified Dakin-West reaction, Angew. Chem. Int. Ed. Engl. 8 (1969) 981, as applied to tripeptides, J. Org. Chem.,50 (1985)1112: reacting a compound of the formula (or alternatively to directly use an amino-terminal-N-alkylated-N-trifluoroacyl-protected tripeptide)



wherein W^1 and W^2 are as defined above, with $(T\text{-}CH_2CO)_2O$ wherein T is halogen, R^4O or R^4S and 4-DMAP, and then further reacting as in method I.

In those cases where the reaction results in a mixture of stereoisomers, these are optionally separated by standard chromatographic or re-crystallisation techniques, and if desired a single stereoisomer is isolated.

DETAILED DESCRIPTION OF THE INVENTION

The following description is illustrative of aspects of the invention.

Synthesis and pharmacy

The chemistry used to prepare the inhibitors claimed here is outlined in the Synthesis schemes (Procedures A to H) and Procedures I and K. The necessary alkoxy- or phenoxy-methyl ketones of arginine were mainly prepared by (I) displacement by R^4O- of the Br of bromomethylketones, either using preformed $NaOR^4$ (Procedure A) or by the use of KF (Procedures D and F) or (II) by alkylation of arginine α-ketol using Ag_2O/R^4I (see Procedure E).

Standard peptide coupling reactions were used to introduce DCha, Pro and their analogues. The carboxyalkyl group on the N-terminus was introduced either by alkylation using bromo-acetates or by Michael addition to tertiary butyl-acrylate (Procedures A, B) or by using pre-incorporation of carboxy-alkyl in the dipeptide moiety (see Procedure K). All protecting groups were then removed (see Deprotection Procedures a-c below).

General experimental procedures:

Standard work-up refers to ethyl acetate extractions, usually washing with 0.3M $KHSO_4$, 1M $KHCO_3$, H_2O and brine followed by filtration through Whatman Phase Separatory paper and drying by toluene azeotroping. TLC was carried out on commercial Merck Silicagel 60F254 coated glass plates. Visualization was by a combination of UV light, heating followed by fluorescamine spray or heating followed by chlorination (Cl_2 tank) and spraying with 1% starch/KI solution. Flash chromatography was carried out on Merck Silicagel 60 (40-63 μm) under pressure of N_2. Amino acid analysis was performed using the Beckman Gold System. Peptides were hydrolysed (6N HCl + phenol at 110°C for 22 h) then injected and the proline peak quantified. MPLC was carried out in glass columns (Anachem) packed with Vydac C18 15-25 μm silica, using gradients of 1% TFA-MeCN into 1% TFA-H_2O with monitoring at 226 nm.

Fractions were analyzed by HPLC and the pure ones pooled and lyophilised. HPLC was carried out using a Spectra-Physics 8700 Series chromatography station. Solvent system as for MPLC with detection at 210 nm. Flow 1.5 ml/min. Column: Novapak C18, 4 μm (8 x 100 mm cartridge, Waters). All intermediates were characterised by NMR (Hitachi-Perkin Elmer R24 60 MHz or Jeol 270 MHz instruments). All final peptides were characterised by their FAB mass spectra (M-Scan Ascot, Berks., U.K.).

Preparation of starting materials:

Boc-Arg(Z_2)-CH_2Br:

(i) Boc-Arg(Z_2)-OH (10 mmol) in dry THF (50 ml) and NMM (10 mmol) was cooled to -10°C and iBC (10

mmol) added dropwise keeping the temp. ¾ -10°C. After 10 min at -10°C the mixed anhydride was poured into CH_2N_2-ether (25 mmol in 150 ml). After 3h excess CH_2N_2 was destroyed with acetic acid and the solution washed with H_2O (3 x) and brine. Drying and evaporation gave the diazoketone as a yellow oil. IR 2100 cm-1 ($\underline{COCHN_2}$).

(ii) The diazoketone (10 mmol) in dry ethyl acetate (200 ml) was cooled to -15°C and 1 M HBr/ethyl acetate (about 11 ml) added dropwise. When the yellow colour was discharged TLC (ethyl acetate/hexane) showed complete conversion of the diazoketone to bromomethyl ketone. The solution was rapidly transferred to a separating funnel and washed with 1 M $KHCO_3$, brine, dried and evaporated to leave a solid. Dissolution in hot EtOH and cooling gave the bromomethyl ketone as an amorphous white powder.

NMR ($CDCl_3$): δ 1.3 (5, 9H), 1.5-1.85 (m, 4H), 3.75-3.95 (m + s, 4H), 4.3 (m, 1H), 5.05 (s, 2H), 5.15 (s, 2H), 5.65 (d, 2H), 7.30 (m, 10H), 9.2 (br, s, 1H), 9.3 (br, s, 1H). Melting point: Softens at 50°C then slowly decomposed >70°C.

Boc-DCha-X-ONSu (X = Pro, Pic or Aze):

(i) Boc-DCha-OH (10 mmol) in CH_2Cl_2/DMF (1:5 50 ml) was treated with HONSu (11 mmol), cooled to 0°C and WSCDI (13 mmol) added. After 30 min it was warmed to room temperature. TLC after 3h showed complete formation of Boc-DCha-ONSu. Addition of Et_2O (200 ml) and washing with H_2O (3 x), brine, drying gave the ester as a colourless foam.

(ii) The N-hydroxysuccinimido ester (10 mmol) in CH_2Cl_2 (50 ml) was treated with H-Pro-OBzl.HCl or H-Pic-OBzl.HCl or H-Aze-OBzl (11 mmol) and iPr_2NEt (20 mmol). After stirring for 3h, standard ethyl acetate/0.3 M $KHSO_4$ work-up gave the dipeptide ester which was pure enough to be used in the next step.

(iii) The Boc-DCha-X-OBzl in THF was hydrogenated over 5% Pd/C at STP for 4h. Filtration and evaporation gave the acid as a solid or foam. Re-crystallisation (iPr_2O or Et_2O/hexane) gave the pure products.

Boc-DCha-Pro-OH (solid m.p. 163 - 166°C): NMR ($CDCl_3$) δ 0.8-2.05 (m, + s at 1.4, 26H), 3.4 (m, 1H), 3.85 (m, 1H), 4.5 (m, 2H), 5.2 (m, 1H).

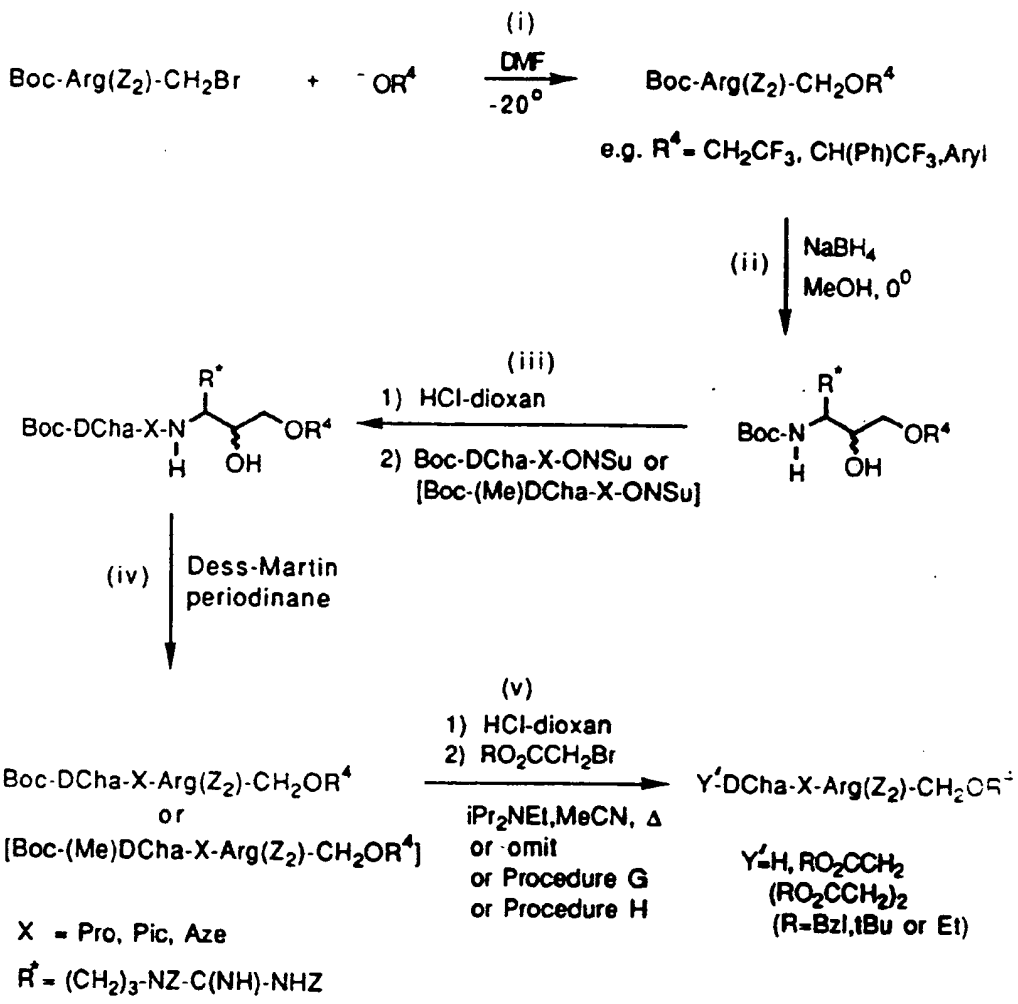
Boc-DCha-Pic-OH (solid m.p. 121-122°C): NMR ($CDCl_3$) δ 0.8-2.05 (m, + s at 1.45, 28H), 3.35 (m, 1H), 3.95 (m, 1H), 4.6-4.9 (m, 1H), 5.4 (m, 1H), 5.6 (m, 1H), 8.8 (br, s, 1H).

Boc-(Me)DCha-Pro-ONSu:

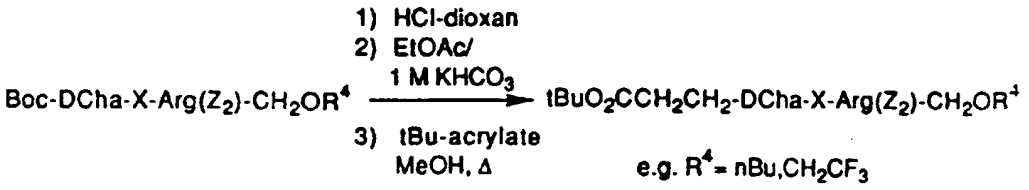
Boc-(Me)DPhe-OH was hydrogenated over 5% Rh-C in 90% acetic acid-H_2O at 0.41 MPa for 3 days giving quantitative yield of Boc-(Me)DCha-OH. Boc-(Me)DCha-OH (10 mmol) and NMM (10 mmol) in CH_2Cl_2 (50 ml) was cooled to -15°C and Ph_2PO-Cl (10 mmol) added. After 20 min, H-Pro-OBzl.HCl (11 mmol) and NMM (20 mmol) was added. After 1h it was allowed to warm to room temperature. After 2h standard work-up and flash chromatography (40% ethyl acetate/hexane) gave pure Boc-(Me)DCha-Pro-OBzl as a colourless oil (80%). NMR ($CDCl_3$): δ 0.5-2.2 (m, + s at 1.4, 26H) 2.65 (s, 3H), 3.5 (m, 2H), 4.3-5.0 (m, 2H), 5.1 (s, 2H), 7.30 (s, 5H). This was converted into the N-hydroxysuccinimido ester as described for Boc-DCha-X-ONSu below.

Synthesis schemes:

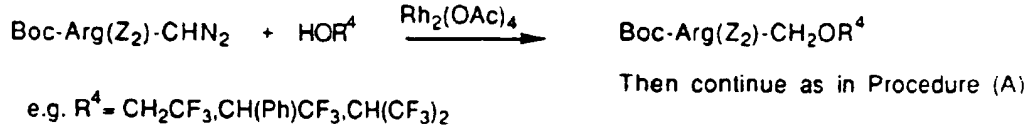
Procedure (A)



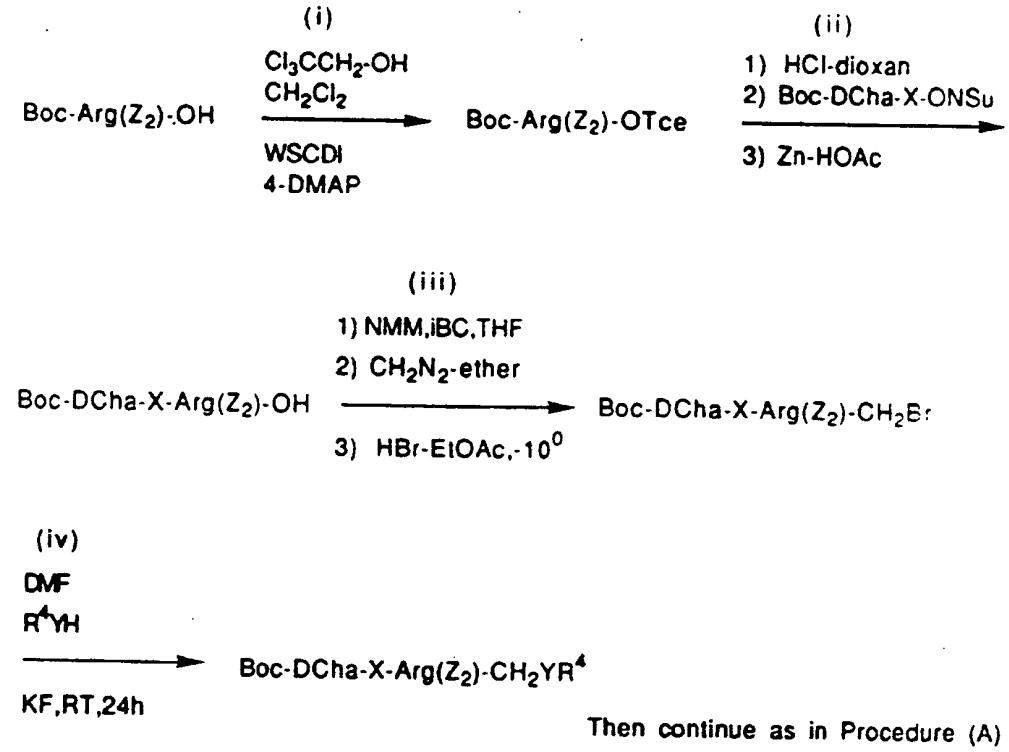
Procedure (B)



Procedure (C)



Procedure (D)



Boc-Arg(Z_2)-OH → Boc-Arg(Z_2)-OTce → Boc-DCha-X-Arg(Z_2)-OH → Boc-DCha-X-Arg(Z_2)-CH_2Br → Boc-DCha-X-Arg(Z_2)-CH_2YR^4

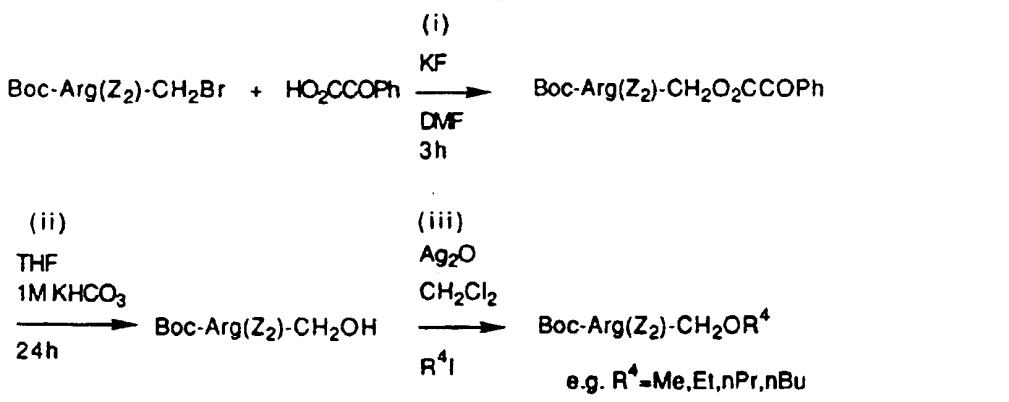
Then continue as in Procedure (A)

Y= O,S

R^4= nBu,Aryl

For Example 1: R^4YH=HO_2CCOPh

Procedure (E)



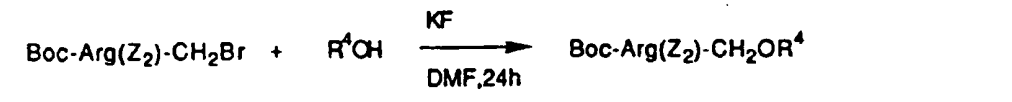
Boc-Arg(Z_2)-CH_2Br + HO_2CCOPh → Boc-Arg(Z_2)-CH_2O_2CCOPh

Boc-Arg(Z_2)-CH_2OH → Boc-Arg(Z_2)-CH_2OR^4

e.g. R^4=Me,Et,nPr,nBu

Then continue as in Procedure (A)

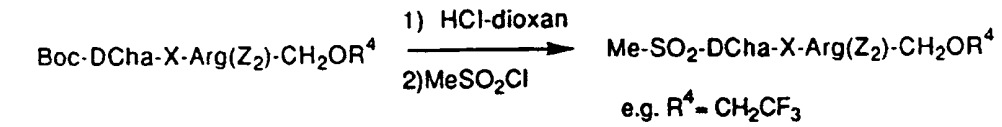
Procedure (F)



e.g R^4 = CH_2CF_3, $CH_2(CF_2)_2CF_3$, Ph(4-OMe)

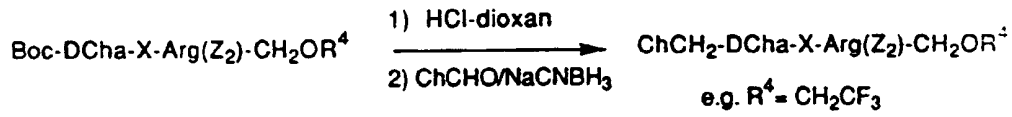
Then continue as in Procedure (A)

Procedure (G)



e.g. R^4 = CH_2CF_3

Procedure (H)



e.g. R^4 = CH_2CF_3

Preparation procedures:

The following preparation procedures illustrate the above methods I-III as well as subsequent steps to final compounds.

Procedure (A)

(i) Boc-Arg(Z_2)-CH_2Br (10 mmol) was added as a solid to a preformed solution of the alkoxide or phenoxide (alcohol or phenol 10 mmol and 80% NaH-oil, 10 mmol) in DMF (40 ml) at -20°C under N_2. After 30 min the solution was warmed to room temperature. 2 hours later 0.3 M $KHSO_4$ was added to neutralize any alkoxide remaining and the DMF removed under vacuum. The crude product was partitioned between ethyl acetate and H_2O, the ethyl acetate layer washed with brine, dried and evaporated. Flash chromatography or crystallisation gave the pure alkoxyketones.

Boc-Arg(Z_2)-CH_2OPh (solid): NMR ($CDCl_3$): δ 1.41 (s, 10H), 1.64 - 1.68 (m, 3H), 3.92 (dd, 2H), 4.5 (m, 1H), 4.62 (q, 2H), 5.1 (s, 2H), 5.2 (s, 2H), 5.5 (d, 1H), 6.8 (d, 2H), 6.95 (t, 1H), 7.2-7.45 (m, 12H), 9.2 (br, s, 1H), 9.3 (br, s, 1H). Melting point 115-118°C.

Boc-Arg(Z_2)-$CH_2OCH_2CF_3$ (solid): NMR ($CDCl_3$): δ 1.35 (s, 10H), 1.55-1.75 (m, 3H), 3.7 (q, 2H), 3.85 (m, 2H), 4.2 (q + m, 3H), 5.05 (s, 2H), 5.15 (s, 2H), 5.7 (d, 1H), 7.15-7.35 (m, 10H), 9.15 (br, s, 1H), 9.3 (br, s, 1H). Melting point 87-90°C.

(ii) The alkoxymethyl or phenoxymethyl ketone in MeOH/THF (1:1) at 0°C was treated with $NaBH_4$ (1 equiv.). After 10 min, 0.3 M $KHSO_4$ was added to pH 7 and the mixture evaporated to remove MeOH/THF. Ethyl acetate was added and after standard work-up (ethyl acetate/0.3 M $KHSO_4$) the alcohol was isolated as a diastereomeric mixture.

(iii) The alcohol was treated with 4M HCl in dioxan for 15 min at room temperature and evaporated. The residue in CH_2Cl_2 (1 mmol in 5 ml) was treated with Boc-DCha-X-ONSu (1 equiv.) and iPr_2NEt (to pH 9 on wet pH paper). After 3h, standard work-up gave the modified tripeptide which was purified by flash chromatography (ethyl acetate-hexane mixtures containing 1% acetic acid). Yield: 50-85%.

(iv) The tripeptide alcohol in CH_2Cl_2 was treated with Dess-Martin periodinane (3 equiv.) (Dess, D.B. and Martin, J.C. J. Org. Chem. 1983, 48. 4155-4156). After 2h stirring at room temperature, standard work-up (Et_2O/1 M $KHCO_3/Na_2S_2O_3$) gave the crude tripeptide ketones which were purified by flash chromatography (ethyl acetate-hexane).

(v) The ketone was treated with 4 M HCl-dioxan for 15 min at room temperature and evaporated. The residue in dry MeCN (1 mmol in 5ml) was treated with benzylbromoacetate or tertiary butyl bromoacetate (1.2 equiv.) and iPr_2NEt (3 equiv.). After reflux for 2h the solution was evaporated and flash columned (ethyl acetate-hexane) giving the benzyloxycarbonylmethyl or tertiary butyloxycarbonylmethyl peptides as oils (40-50%).

In Examples 30 and 31, 2.5 equiv. of the bromoacetate were used to achieve bis-alkylation.

Procedure (B)

The peptide alkoxymethylketone was treated with excess 4 M HCl-dioxan for 15 min at room temperature. Evaporation gave the HCl salt which was partitioned between ethyl acetate and 1 M $KHCO_3$. The ethyl acetate was separated, dried and evaporated giving the free amine which was taken up in MeOH and freshly distilled tertiary butylacrylate added (1.5 equiv.). Reflux for 4h gave the tertiary butoxycarbonylethyl peptide which was purified by flash chromatography in ethyl acetate/hexane.

Procedure (C)

Boc-Arg(Z_2)-CHN_2 (1 mmol) dissolved in the alcohol R^4OH (5 ml) was treated with $Rh_2(OAc)_4$ (cat.). After several hours at room temperature, TLC analysis showed no diazoketone remaining. The alcohol was removed in vacuo and the product isolated by flash chromatography using ethyl acetate/hexane mixtures.

Procedure (D)

(i) Boc-Arg(Z_2)-OH (10 mmol) in dry CH_2Cl_2 (50 ml) was treated with 2,2,2-trichloroethanol (11 mmol) and 4-DMAP (1 mmol), cooled to 0°C and WSCDI (13 mmol) added. After 30 min, it was allowed to warm to room temperature and stirred for 24h. Evaporation and partition between ethyl acetate/0.3 M $KHSO_4$, followed by 3 x washes with 0.3 M $KHSO_4$, 1 x H_2O, 1 x brine, drying and evaporation gave the Tce ester which was used as such.

(ii) The Tce ester (10 mmol) was treated with 4 M HCl-dioxan (50 ml) for 20 min at room temperature and then evaporated. After drying, the residue in CH_2Cl_2 (50 ml) was treated sequentially with Boc-DCha-X-ONSu (10 mmol) (X = Pro, Pic) and iPr_2NEt (to pH 9 on wet pH paper). After 3h, standard work-up (ethyl acetate/0.3 M $KHSO_4$) gave the tripeptide ester as an oil. The Tce ester (10 mmol) in 90% acetic acid-H_2O (50 ml) was treated at 5 min intervals with small portions of freshly activated Zn over 1h. After a further 1h, the mixture was filtered and the solution evaporated. Standard work-up (ethyl acetate/0.3 M $KHSO_4$) gave the tripeptide acid which was purified by flash chromatography on silica (2% acetic acid-ethyl acetate) giving the tripeptide acid as a colourless foam (80% over 3 steps).

(iii) The tripeptide acid was converted into the bromomethyl ketone using the same procedure as that described for Boc-Arg(Z_2)-CH_2Br. The tripeptide bromomethyl ketone was obtained as a colourless oil by flash chromatography using ethyl acetate/hexane mixtures.

NMR ($CDCl_3$): δ 0.9 (m), 1.15 (m), 1.25 (m), 1.35 (s), 1.6 (m), 1.85 (m), 2.1 (m) [total 30H], 3.3 (m, 1H), 3.7 (m, 1H), 3.95 (m, 2H), 4.15 (s, 2H), 4.25 (m, 1H), 4.4 (m, 1H), 4.5 (m, 1H), 5.0 (d, 1H), 5.1 (dd, 2H), 5.2 (s, 2H), 7.2-7.4 (s, m, 10H), 9.2-9.5 (2 br, s, 2H).

(iv) The tripeptide bromomethyl ketone (1 mmol) in dry DMF (5 ml) was treated with fluorinated alcohol, phenol or thiol (1.2 mmol) and anhydrous potassium fluoride (1.5 mmol) and stirred at room temperature for 24h. Evaporation followed by standard work-up and flash chromatography gave the tripeptide ketones.

Boc-DCha-Pro-Arg(Z_2)-CH_2O-Ph(4-Me): NMR ($CDCl_3$) δ 0.9 (m), 1.15 (m), 1.25 (m), 1.35 (s), 1.6 (m), 1.85 (m), 2.1 (m) [total 30H], 2.38 (s, 3H), 3.4 (m, 1H), 3.9 (m, 1H), 4.1 (br, s, 2H), 4.4 (m, 1H), 4.6 (m, 1H), 4.7 (m, 1H), 4.9 (q, 2H), 6.9 (d, 1H), 7.15 (d, 1H), 7.4-7.5 (m, 2H), 7.45 (s, 10H), 9.4 (br, s, 1H), 9.6 (br, s, 1H).

For Example 37 the protected sulphide was oxidised to the sulphone using m-chloroperbenzoic acid in dichloromethane at room temperature.

Procedure (E)

(i) Boc-Arg(Z_2)-CH_2Br (10 mmol) and benzoylformic acid (12 mmol) in DMF (40 ml) were treated with KF (14 mmol). After stirring for 3h the DMF was evaporated and the product partitioned between ethyl acetate/H_2O. Drying and evaporation gave the crude benzoylformate ester which was purified by crystallisation (CH_2Cl_2-hexane) giving the product as a white solid (86%).

NMR ($CDCl_3$): δ 1.4 (s, 9H), 1.65-1.9 (m, 4H), 3.95 (m, 2H), 4.3 (m, 1H), 4.95 (q, 2H), 5.15 (ABq, 2H),

5.25 (s, 2H), 5.9 (d, 1H), 7.35 (m, 10H), 7.5 (t, 2H), 7.65 (t, 1H), 8.15 (t, 2H), 9.25 (br, s, 1H), 9.45 (br, s, 1H). Melting point 130-132°C.

(ii) The benzoylformate ester (5 mmol) in THF (200 ml) and 1 M $KHCO_3$ (200 ml) was stirred vigorously at room temperature for 24h. The THF was separated and evaporated and the aqueous phase extracted with ethyl acetate which was combined with the material from the THF. Crystallisation from CH_2Cl_2-hexane gave the α-ketol as a white solid (90%).

NMR ($CDCl_3$): δ 1.4 (s, 9H), 1.7 (m, 4H), 2.95 (t, 1H), 3.95 (m, 2H), 4.25 (m, 2H), 5.15 (s, 2H), 5.25 (s, 2H), 5.6 (d, 1H), 7.35 (m, 10H), 9.25 (br, s, 1H), 9.4 (br, s, 1H). Melting point 101-103°C.

(iii) The α-ketol (1 mmol) in dry CH_2Cl_2 (5 ml) was treated with alkyl iodide (5 to 10 mmol) and silver oxide (2 mmol). The mixture was refluxed in the dark for 2 to 17 hours (e.g. MeI, EtI, nPrI: 2h; nBuI: 5h). Evaporation followed by flash chromatography (ethyl acetate-hexane) gave the alkoxymethyl ketones as colourless oils (50-85%).

Boc-Arg(Z_2)-CH_2OEt (oil): NMR ($CDCl_3$): δ 1.15 (t, 3H), 1.4 (s, 9H), 1.5-1.8 (m, 4H), 3.4 (q, 2H), 3.95 (t, 2H), 4.1 (q, 2H), 4.45 (m, 1H), 5.15 (s, 2H), 5.25 (s, 2H), 5.4 (d, 1H), 7.35 (m, 10H), 9.25 (br, s, 1H), 9.4 (br, s, 1H).

Boc-Arg(Z_2)-CH_2NBu (oil): NMR ($CDCl_3$): δ 0.9 (t, 3H), 1.25-1.8 (m) + 1.4 (s) [17H], 3.3 (dd, 2H), 3.95 (t,2H), 4.05 (q, 2H), 4.45 (m, 1H), 5.1 (s, 2H), 5.2 (s, 2H), 5.35 (d, 1H), 7.35 (m, 10H), 9.25 (br, s, 1H), 9.4 (br, s, 1H).

Procedure (F)

Boc-Arg(Z_2)-CH_2Br was treated with CF_3CH_2OH, $CF_3(CF_2)_2CH_2OH$ or Ar-OH and KF in DMF using the procedure outlined in Procedure (D) (iv).

Procedure (G)

The Boc protected peptides were treated with 4 M HCl-dioxan for 15 min at room temperature and evaporated. The residue in CH_2Cl_2 was treated with $MeSO_2Cl$ (1.1 equiv.) and iPr_2NEt (2.5 equiv.). After 1h standard work-up and flash-chromatography gave the methylsulphonylpeptides which were deprotected as in Deprotection procedure (a).

Procedure (H)

The peptides were Boc deprotected as above and the HCl washed out using ethyl acetate/1 M $KHCO_3$ partition. The free amines in MeOH cooled to 0°C were treated with the aldehyde Ch-CHO (1.5 equiv.) and $NaCNBH_3$ (1 equiv.). After 1 hour evaporation in the cold and flash chromatography gave the N-alkylated peptide. Deprotection using Deprotection procedure (a).

Procedure (I)

Boc-(3-trans-phenyl)-D,Lproline was prepared as described in Chung et al. J. Org. Chem. 1990, 55, 270, and coupled to H-Pro-OBzl as described above for Boc-(Me) DCha-Pro-OBzl. The dipeptide was then converted to its -ONSu ester as described below.

Boc-(3-trans-cyclohexyl)-D,Lproline was prepared from the phenyl analogue by hydrogenation over 5% Rh-C in 90% HOAc-H_2O at 0.41 MPa for 3 days.

Procedure (K)

Synthesis of the intermediate N-($BzlO_2C$-CH_2-),N-(CF_3CO)-DCha-Pro-ONSu

(i) H_2 -Pd/C

(ii)$BzlO_2C$-CHO,

$$\text{Z-DCha-Pro-OtBu} \xrightarrow{NaCNBH_3^-} \text{N-(BzlO}_2\text{C-CH}_2\text{-),N-(CF}_3\text{CO)-DCha-Pro-ONSu}$$

(iii) $(CF_3CO)_2O$

(iv) TFA

(v) HONSu, WSCDI

(i) Z-DCha-Pro-OtBu (made by standard peptide coupling reactions) was hydrogenated in THF over 5% Pd-C at standard temperature and pressure for 24 h. Filtration and evaporation provided H-DCha-Pro-OtBu (oil, 100%).

(ii) The previous product (2 mmol) and benzyl glyoxylate (1 equivalent) in benzene were subjected to three evaporations (fresh benzene added each time) to remove water. The residual imine (2 mmol) in 1% acetic acid/methanol (8 ml) was treated with $NaCNBH_3$ (2 mmol). After 1 h, evaporation followed by flash chromatography on silica (60% EtOAc-hexane) gave $BzlO_2CCH_2$-DCha-Pro-otBu, 385 mg (41%).

(iii) The above product (380 mg) in dry CH_2Cl_2 (8 ml) was treated with Et_3N (2 equivalents) and $(CF_3CO)_2O$ (1.2 equivalents). After 40 min, evaporation and flash chromatography (silica, 30% EtOAC-hexane) gave the N-($BzlO_2C$-CH_2-), N-(CF_3CO)-DCha-Pro-OtBu as an oil, 390 mg (86%).

^{1}HNmr($COCl_3$) - complex due to presence of 4 rotamers - eg. tBu group at δ 1,4-1.5 was split four times in the ratio 1:0.25:0.8:0.4. Ù 0,9(m), 1.1(m), 1.65(m), 1.95(m), 2.2(m) [17H]; 1.4-1.5 [4xs, 9H]; 3.1 (m), 3.5(m), 3.7(m)[2H]; 4.3-4.6 [m,3H]; 5.05-5.4 (m,3H); 7.35 [,s,5H].

(iv) The above product (335 mg) was treated with CH_2Cl_2-TFA (1:1,8 ml)for 2.5 h at room temperature. Evaporation followed by three evaporations from toluene gave the free acid, 100%.

(v) The previous acid was converted to its -ONSu ester (100%) using HONSu as described previously for Boc-DCha-OH.

The intermediate may be coupled to H-Arg$(Z)_2$-CH_2-Y-R^4 using the general methods already outlined. Deprotection procedure a provides the peptide protected with N-CF_3-CO-. The N-CF_3-CO is removed by deprotection procedure d.

Deprotection procedures:

(a) The protected peptide in MeOH / H_2O (3:1) containing 1M HCl (2 equivalents) was hydrogenated over 5% Pd/C at STP for 40 min. Filtration (0.2 μm) and evaporation was followed by lyophilization from water to give the peptides as fluffy white solids. Purification, if required, was carried out by MPLC (see general procedures).

(b) The protected peptide was first treated with TFA/CH_2Cl_2 (1:1) for 1 hour and evaporated, then hydrogenated as above under (a).

(c) The COCOPh group was first hydrolysed as in (E)(ii) then hydrogenated over H_2-Pd/C as in (a).

(d) Removal of N-CF_3-CO (N-trifluoroacetyl):

The N-trifluoroacetyl peptide was dissolved in MeCN-H_2O-0.880 ammonia (1:1:1) and kept at room temperature for 24 h. Evaporation followed by purification if necessary provided the peptide.

Examples

The following examples illustrates the principles of the invention in more detail.

Examples of compounds of the invention are listed in Table 1, Table 2 indicates the procedures used in their preparation and described in the section titled "Preparation procedures", and Table 3 presents characterising data for the compounds listed.

Examples 1-37

Table 1

Example No.	Formula
1	H-DCha-Pro-D,LArg-CH_2-OH
2	H-DCha-Pro-D,LArg-CH_2-O-Me
3	H-DCha-Pro-Arg-CH_2-O-CH_2-CF_3
4	H-DCha-Pro-Arg-CH_2-O-$CH(CF_3)_2$
5	H-DCha-Pro-Arg-CH_2-O-$C^*H(Ph)$-CF_3
6	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-CH_2-CF_3
7	Et-OOC-CH_2-DCha-Pro-D,LArg-CH_2-O-CH_2-CF_3
8	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-CH_2-CF2-CF2-CF_3
9	HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph
10	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(4-OMe)
11	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(4-tBu)
12	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(4-Me)
13	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(4-F)
14	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(3-F)
15	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(2-F)
16	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(3-CF_3)
17	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(4-CF_3)
18	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Ph(2-CF_3)
19	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-C_6F_5
20	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-O-Et
21	HOOC-CH_2-DCha-Pro-Arg-CH_2-O-nPr
22	HOOC-CH_2-DCha-Pro-Arg-CH_2-O-nBu
23	HOOC-CH_2-DCha-Pro-Arg-CH_2-O-iBu
24	HOOC-CH_2-DCha-Aze-D,LArg-CH_2-O-CH_2-CF_3
25	HOOC-CH_2-DCha-Pic-D,LArg-CH_2-O-CH_2-CF_3
26	HOOC-CH_2-DCha-Pic-Arg-CH_2-O-nBu
27	Me-DCha-Pro-D,LArg-CH_2-O-CH_2-CF_3
28	Me-SO2-DCha-Pro-D,LArg-CH_2-O-CH_2-CF_3
29	Ch-CH_2-DCha-Pro-D,LArg-CH_2-O-CH_2-CF_3
30	$(HOOC-CH_2)_2$-DCha-Pro-D,LArg-CH_2-O-CH_2-CF_3

Table 1 (continued)

Example No.	Formula
31	$(HOOC-CH_2)_2$-DCha-Pro-Arg-CH_2-O-iBu
32	HOOC-CH_2-CH_2-DCha-Pro-D,LArg-CH_2-O-nBu
33	HOOC-CH_2-CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3
34	H-(3-trans-Ph)-D,LPro-D,LArg-CH_2-O-CH_2-CF_3
35	H-(3-trans-Ch)-D,LPro-D,LArg-CH_2-O-CH_2-CF_3
36	HOOC-CH_2-DCha-Pro-Arg-CH_2-S-nBu
37	HOOC-CH_2-DCha-Pro-D,LArg-CH_2-SO2-nBu

* absolute configuration R or S

Table 2

Example No.	Preparation procedure	Deprotection procedure
1	D	c
2	E	b
3	A or C	b
4	A or C	b
5	A or C	b
6	A	a
7	A	a
8	F	a
9	A or D	a
10	F	a
11	D	a
12	D	a
13	D	a
14	D	a
15	D	a
16	D	a
17	D	a
18	D	a
19	D	a
20	E	a
21	E	a
22	E	a
23	E	a
24	A	a
25	A	a
26	E	a
27	A and F	a
28	A and G	a
29	A and H	a
30	A	a

Table 2 (continued)

Example No.	Preparation procedure	Deprotection procedure
31	E	a
32	E and B	b
33	A and B	b
34	I and A	b
35	I and A	b
36	D	a
37	D	a

Table 3

Example No.	Mol. Wt	FAB MS (M + 1)	AAA: Peptide Content (%)*	HPLC retention** time (min)/system
1	438.57	439	56/Pro	10.3 /E
2	452.60	453	65/Pro	9.9 /E
3	520.6	521	59/Pro	8.6 /A
4	588.6	589	60/Pro	10.7 /A
5	596.7	597	61/Pro	12.2 /A
6	578.64	579	72/Pro	14.8 /C
7	606.69	607.7	65/Pro	22.0 /B
8	678.65	679.8	76/Pro	18.2 /G
9	572.71	573.3	73/Pro	19.9 /B
10	602.74	603.8	76/Pro	13.4 /G
11	628.82	629.5	77/Pro	22.0 /G
12	586.74	587.2	75/Pro	16.0 /G
13	590.70	591.4	76/Pro	15.2 /G
14	590.70	591.4	63/Pro	15.0 /G
15	590.70	591.4	54/Pro	14.0 /G
16	640.71	641.5	68/Pro	19.0 /G
17	640.71	641.6	70/Pro	20.0 /G
18	640.71	641.4	65/Pro	18.4 /G
19	662.66	663	49/Pro	21.0 /G
20	524.67	525.4	79/Pro	15.5 /B
21	538.69	539	67/Pro	9.2 /F
22	552.72	553	69/Pro	19.3 /B
23	552.72	553.4	56/Pro	9.2 /F
24	564.61	565	67/Aze	13.6 /D
25	592.66	593	90/Pic	8.4 /D
26	566.75	567.4	86/Pic	17.0 /G
27	534.63	535.6	70/Pro	18.8 /B
28	598.69	599	71/Pro	11.2 /D

Table 3 (continued)

Example No.	Mol. Wt	FAB MS (M + 1)	AAA: Peptide Content (%)*	HPLC retention** time (min)/system
29	616.77	617	64/Pro	14.5 /C
30	636.67	637.6	70/Pro	12.0 /A
31	610.76	611.2	67/Pro	10.0 /F
32	566.75	567.5	65/Pro	19.7 /B
33	592.66	593	46/Pro	10.6 /F
34	540.59	541	56/Pro	14.3 /C
35	546.64	547	49/Pro	15.9 /C
36	568.78	569.4	45/Pro	17.4 /G
37	600.78	601.3	62/Pro	14.6 /G

*Based on amino acid as indicated

**See General experimental procedures. Times are given for L-Arg epimers. D-epimers (minor) usually run about 0.5 min earlier.

System A: 20% increased to 80% of 1% TFA-MeCN into 1% TFA-H_2O over 25 min. (20-80%, 25 min)

System B: 10-60%, 30 min
System C: 10-90%, 30 min
System D: 30-100%,30 min
System E: 10-90%, 20 min
System F: 20-100%,20 min
System G: 20-70%, 30 min

Example 38

Solution for continuous intravenous administration
A solution is prepared from the following ingredients:

Thrombin inhibitor	50 mg
Sodium chloride for injection	4.5 g
Water for injection up to 500 ml	

The active constituent and the sodium chloride are dissolved in the water whereafter the solution is filtered and then sterilised by autoclaving or by filtration through a sterile 0.2 µm filter and aseptically filled into sterile infusion bottles.

Example 39

Solution for injection.
A solution is prepared from the following ingredients:

Thrombin inhibitor	5 g
Sodium chloride for injection	9 g
Water for inj. up to 1000 ml	

The active constituent and the sodium chloride are dissolved in the water whereafter the solution is filtered and then sterilised by autoclaving or by filtration through a sterile 0.2 µm filter and aseptically filled into sterile ampoules (5 ml).

Example 40

Solution for nasal administration
A solution is prepared from the following ingredients:

Thrombin inhibitor	10 g
Glycerol	200 g
Methyl p-hydroxybenzoate	1 g
Propyl p-hydroxybenzoate	0.2 g
Water for inj. up to 1000 ml	

The active constituent and the preservatives were dissolved in glycerol and the main part of the water. The volume is then adjusted to 1000 ml and the solution is filled into sterile polyethylene containers.

Example 41

Tablets for oral administration
1000 tablets are prepared from the following ingredients:

Thrombin inhibitor	100 g
Lactose	200 g
Polyvinyl pyrrolidone	30 g
Microcrystalline cellulose	30 g
Magnesium stearate	6 g

The active constituent and lactose are mixed with an aqueous solution of polyvinyl pyrrolidone. The mixture is dried and milled to form granules. The microcrystalline cellulose and then the magnesium stearate are then admixed. The mixture is then compressed in a tablet machine giving 1000 tablets, each containing 100 mg of active constituent.

Example 42

Gelatine capsules for oral administration
Gelatine capsules are filled with a mixture of the following ingredients:

Thrombin inhibitor	50 mg
Magnesium stearate	3 mg
Lactose	100 mg

Biology

Determination of thrombin clotting time and $IC_{50}TT$:

Human thrombin (T 6769, Sigma Chem Co) in buffer solution, pH 7.4, 100 µl, and inhibitor solution, 100 µl, are incubated for one min. Pooled normal citrated human plasma, 100 µl, is then added and the clotting time measured in an automatic device (KC 10, Amelung).

The clotting time in seconds is plotted against the inhibitor concentration, and the $IC_{50}TT$ is determined by interpolation.

$IC_{50}TT$ is the concentration of inhibitor that doubles the thrombin clotting time for human plasma. $pIC_{50}TT$ is the -log 10 of $IC_{50}TT$ in mol/l. The results are presented in Table 4.

Table 4

Example No.	$pIC_{50}TT$
1	7.71
2	7.81
3	7.92
4	7.38
5	7.77
6	8.04
7	7.70
8	7.64
9	8.45
10	7.90
11	7.86
12	8.25
13	8.22
14	8.15
15	8.12
16	7.77
17	8.68
18	7.30
19	8.27
20	8.17
21	8.14
22	8.69
23	7.64
24	8.01
25	8.00
26	7.89
27	7.52
28	6.85
29	6.47
30	7.38

Table 4 (continued)

Example No.	$pIC_{50}TT$
31	6.88
32	7.63
33	7.78
34	7.03
35	7.25
36	7.57
37	7.72

ABBREVIATIONS

4-DMAP =	4-dimethylamino pyridine
AAA =	amino acid analysis
Arg =	L-arginine
$Arg(Z_2)$ =	ÙN, N-dibenzyloxycarbonyl-L-arginine
Aze =	L-azetidine-2-carboxylic acid
Boc =	tertiary butoxy carbonyl
Bu =	butyl
Bzl =	benzyl
Ch =	cyclohexyl
Cha =	L-ß-cyclohexylalanine
DMF =	dimethyl formamide
Et =	ethyl
EtOAc =	ethyl acetate
FAB =	fast atom bombardment
FI to FXIII =	coagulation factors I to XIII
FII_a to $FXIII_a$ =	activated form of coagulation factors II to XIII
Gly =	glycine
HMW-K =	high molecular weight kininogen
HOAc =	acetic acid
HONSu =	N-hydroxysuccinimide
HPLC =	high performance liquid chromatography
iBC =	isobutyl chloroformate
Kall =	kallikrein
Me =	methyl
MPLC =	medium pressure liquid chromatography
NMM =	N-methyl morpholine
Nph =	naphthyl
Ph =	phenyl
Pic =	L-pipecolinic acid

PL =	phospholipids
Pr =	propyl
Prekall =	prekallikrein
Pro =	L-proline
STP =	standard temperature and pressure
Tce =	2,2,2-trichloroethyl
TFA =	trifluoracetic acid
THF =	tetrahydrofuran
Val =	L-valine
WSCDI =	water soluble carbodiimide
Z =	benzyloxy carbonyl

Prefixes n, i and t have their usual meanings: normal, iso and tertiary.

SEQUENCE LISTING

NUMBER OF SEQUENCES: 1

(1) INFORMATION FOR SEQ ID NO:1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..20

(D) OTHER INFORMATION: /note= "Peptide sequence containing thrombin cleavage site in the human fibrinogen A-alpha chain."

(ix) FEATURE:

(A) NAME/KEY: Cleavage-site

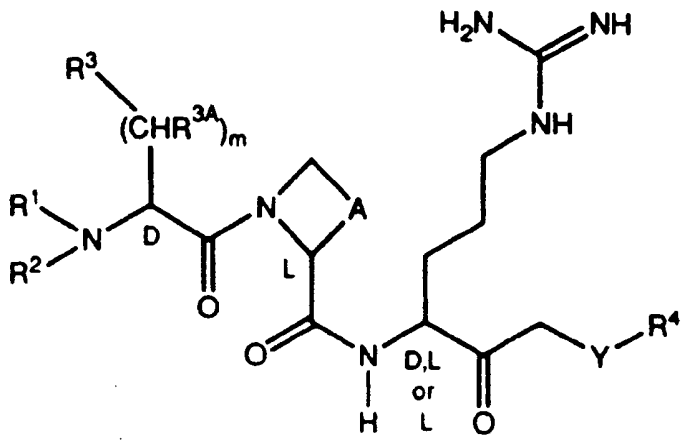
(B) LOCATION: 16..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Asp Ser Gly Glu Gly Asp Phe Leu Ala Glu Gly Gly Gly
1               5                  10
Val Arg Gly Pro Arg Val
15                  20
```

Claims

1. A compound of the general formula



1

wherein:

A represents $-CH_2-$, $-CH=CH-$, $-CH_2-CH_2-$ or $-CH_2-CH_2-CH_2-$;

R^1 and R^2 are the same or different and each represents H or X-B-, where B is a straight or branched alkylene group having 1-3 carbon atom and X is H, methyl, ethyl, a cycloalkyl group having 3-6 carbon atoms or R'CO-, where R' is OH, a straight or branched alkoxy group having 1-4 carbon atoms, NH_2 or NHR", where R" is a straight or branched alkyl group having 1-4 carbon atoms, or X is a carboxylic acid mimic, known per se, selected from $-PO(OR"')_2$, $-SO_3H$ and 5-(1H)-tetrazolyl, and R"' is H, methyl or ethyl, or B is $-SO_2-$ and X is methyl or ethyl;

m is 0, 1 or 2, R^3 represents a cyclohexyl group and R^{3A} represents H; or

m is 1 and R^3 represents a cyclohexyl or phenyl group and R^{3A} forms an ethylene bridge together with R^1 ;

Y represents O or $S(O)_p$, where p is 0, 1 or 2;

R^4 represents H; a straight or branched alkyl or a cycloalkyl having 1 to 6 carbon atoms unsubstituted or substituted with one or more fluoro atoms and/or substituted with a phenyl group; a substituted or unsubstituted aromatic ring selected from phenyl, 4-methoxy-phenyl, 4-tertiary-butyl-phenyl, 4-methyl-phenyl, 2-, 3- or 4-trifluoro-methyl-phenyl, phenyl substituted with 1-5 fluoro atoms; or $-CH(CF_3)$-phenyl, either as such or in the form of a physiologically acceptable salt and including stereoisomers.

2. A compound according to claim 1 wherein

A represents $-CH_2-CH_2-$ or $-CH_2-CH_2-CH_2-$;

R^1 is H and R^2 represents $HOCO(CH_2)_n-$ or $CH_3CH_2OCO(CH_2)_n-$, and n is 1 or 2;

Y is O

m is 1, R^3 represents a cyclohexyl group and R^{3A} represents H.

3. A compound according to claim 1 selected from

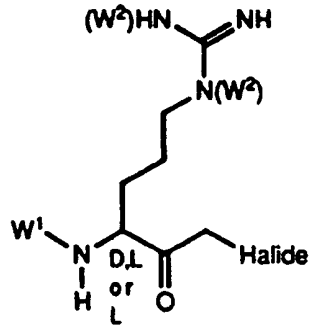
H-DCha-Pro-Arg-CH_2-OH,
H-DCha-Pro-Arg-CH_2-O-Me,
H-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
H-DCha-Pro-Arg-CH_2-O-$CH(CF_3)_2$,
H-DCha-Pro-Arg-CH_2-O-(R or S)CH(Ph)-CF_3,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
Et-OOC-CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF2-CF2-CF_3,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(4-OMe),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(4-tBu),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(4-Me),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(4-F),

HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(3-F),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(2-F),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(3-CF_3),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(4-CF_3),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Ph(2-CF_3),
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-C6F5,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-Et,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-nPr,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-nBu,
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-iBu,
HOOC-CH_2-DCha-Aze-Arg-CH_2-O-CH_2-CF_3,
HOOC-CH_2-DCha-Pic-Arg-CH_2-O-CH_2-CF_3,
HOOC-CH_2-DCha-Pic-Arg-CH_2-O-nBu,
Me-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
Me-SO_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
Ch-CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
(HOOC-CH_2)$_2$-Cha-Pro-Arg-CH_2-O-CH_2-CF_3,
(HOOC-CH_2)$_2$-DCha-Pro-Arg-CH_2-O-iBu,
HOOC-CH_2-CH_2-DCha-Pro-Arg-CH_2-O-nBu,
HOOC-CH_2-CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
H-(3-trans-Ph)-Pro-Arg-CH_2-O-CH_2-CF_3,
H-(3-trans-Ch)-Pro-Arg-CH_2-O-CH_2 -CF_3,
HOOC-CH_2-DCha-Pro-Arg-CH_2-S-nBu and
HOOC-CH_2-DCha-Pro-Arg-CH_2-SO_2-nBu,
either as such or in the form of a physiologically acceptable salt and including stereoisomers.

4. A compound according to claim 1 selected from
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3,
HOOC-CH_2-DCha-Pic-Arg-CH_2-O-CH_2-CF_3,
HOOC-CH_2-DCha-Pic-Arg-CH_2 -O-nBu,
HOOC-CH_2-CH_2-DCha-Pro-Arg-CH_2 -O-nBu,
HOOC-CH_2 -CH_2-DCha-Pro-Arg-CH_2-O-CH_2-CF_3 and
HOOC-CH_2-DCha-Pro-Arg-CH_2-O-nBu,
either as such or in the form of a physiologically acceptable salt and including stereoisomers.

5. A compound according to any of claims 1-4 for use in therapy.

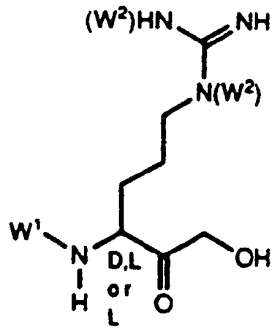
6. A process for preparing a compound according to any of claims 1-4 , which process comprises
(method I) displacement by R^4Y^- (Y=O,S) of the halogen of a halomethylketone of the formula



wherein W^1 is an amino terminal protecting group and W^2 is a protecting group , reduction of the ketone to alcohol, removal of the amino terminal protecting group, standard peptide coupling, followed by oxidation of the alcohol, giving the protected tripeptide ketone, removal of the amino terminal protecting group, followed by N-alkylation, and deprotection, or replacing a protected dipeptide in the coupling reaction referred to above with an amino-terminal-N-alkylated-N-trifluoroacyl-protected dipeptide, followed by oxi-

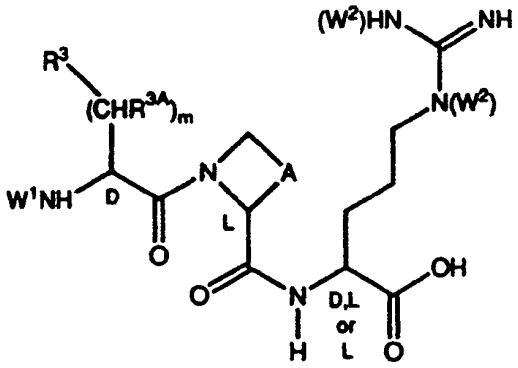
dation and deprotection, or

(method II) alkylation, with an R^4- halide, of an α-ketol of the formula



wherein W^1 and W^2 are as defined above, and then further reacting as in method I, or

(method III) reacting a compound of the formula



wherein W^1 and W^2 are as defined above, with $(T\text{-}CH_2CO)_2O$ wherein T is halogen, R^4O or R^4S and 4-DMAP, and then further reacting as in method I, and if desired forming a physiologically acceptable salt, and in those cases where the reaction results in a mixture of stereoisomers, these are optionally separated by standard chromatographic or re-crystallisation techniques, and if desired a single stereoisomer is isolated.

7. A pharmaceutical preparation comprising an effective amount of any of the compounds outlined in claims 1-4, and furthermore comprising one or more pharmaceutical carriers.

8. Use of compound according to any of claims 1-4 as an active ingredient for manufacture of a pharmaceutical preparation for inhibition of thrombin in a human or animal organism.

9. Use of compound according to any of claims 1-4 as an anticoagulant agent.

10. A method for obtaining inhibition of thrombin in a human or animal organism in need of such inhibition, comprising administering to said organism an inhibitory effective amount of a compound claimed in any of claims 1-4.

11. A method of treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues in a human or animal organism, comprising administering to a host in need of such treatment or prophylaxis an effective amount of a compound claimed in any of claims 1-4.

12. A compound, a process, a pharmaceutical preparation, a use and a method as claimed in any of claims 1-11 and substantially as described.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application number
EP 92850201.2

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	EP-A2-192 135 (BEHRINGWERKE AKTIEN-GESELLSCHAFT) * the whole document * ---	1-8, 12	C07K 5/08 A61K 37/64
A	US-A-4 318 904 (E.N. SHAW ET AL.) * the whole document * ---	1-8, 12	
P,A	EP-A2-479 489 ELI LILLY AND COMPANY) * the whole document * -----	1-8, 12	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C07K
A61K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely:

Claims searched incompletely:

Claims not searched: 9-11

Reason for the limitation of the search:

Methods for treatment of the human or animal body by surgery or therapy, /see art. 52(4) of the European Patent Convention/.

Place of search	Date of completion of the search	Examiner
STOCKHOLM	1 DECEMBER 1992	CARLBORG E.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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